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Short title: Uterine relaxants and embryo transfer

The effect of peri-implantation administration of uterine relaxing agents in assisted reproduction treatment cycles: a systematic review and meta-analysis

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Author biography

Dr Mohammed Khairy Mahmoud graduated in 1998 in Egypt. After finishing his residency programme, he obtained a scholarship to the UK in 2005. He obtained his MD in infertility and reproductive medicine in 2008 on his work on the role of ultrasound markers of ovarian reserve (antral follicle count, ovarian volume and ovarian stromal Doppler) in the prediction of ovarian response in assisted conception cycles. Dr Mahmoud is now a subspecialty trainee

in reproductive medicine at the Birmingham Fertility Centre, Birmingham Women's Hospital. His main area of interest is recurrent implantation failure and interventions for improving endometrial receptivity, embryo selection and embryo transfer.

Abstract

Sub-endometrial junctional zone peristalsis is increased by ovarian stimulation and traumatic embryo transfer, and is linked with decreased implantation and pregnancy rates in assisted reproduction treatments. Various agents have been used to inhibit uterine hyper-peristalsis at the time of embryo transfer with conflicting results. This systematic review aimed to identify if uterine relaxants administered in the peri-implantation period during assisted reproduction treatments could improve pregnancy outcomes through literature search with no language restrictions. The review reports on 3546 patients in 17 randomized controlled trials published between 1993 and 2014. Women undergoing assisted reproduction techniques who either received a uterine relaxant agent in the peri-implantation period versus placebo or no treatment were included. Primary outcome was live birth rate. The meta-analyses did not show statistically significant benefit of any uterine relaxing agents on live birth rate. Other meta-analyses did not show a significant effect on the clinical pregnancy, spontaneous abortion, ectopic pregnancy and multiple pregnancy rate. Most of the included studies were of low quality and lacked significant power to detect minimally important effect. Evidence is insufficient to recommend using these agents in routine practice. Further methodologically robust randomized controlled trials with more refined selection criteria might reveal a beneficial effect.

KEYWORDS: Embryo transfer, assisted reproduction, oxytocin antagonists, prostaglandin synthetase inhibitors, uterine contractions

<A>Introduction

Successful embryo implantation in assisted reproduction technique cycles depends on embryo quality, endometrial receptivity and a non-traumatic embryo transfer. Difficult and traumatic embryo transfer has been strongly linked to lower pregnancy rates (Kovacs, 1999, Schoolcraft *et al*., 2001). It has been shown that difficult embryo transfer stimulates uterine junctional zone contractions (Lesny *et al*., 1998). This has been postulated to lead to non-adherence of the embryo(s) to the endometrium, expulsion of the embryos from the uterine cavity shortly after embryo transfer (Lesny *et al*., 1998; Mansour *et al*., 1990; Lesny and Killick, 2004), or both. Furthermore, it has also been shown in normal fertile women that sub-endometrial peristalsis of the junctional zone myometrium progressively increases during menstrual cycle reaching a peak at time of ovulation with the pattern changing from predominantly ante-grade (fundo-cervical) in the menstrual and proliferative phase to retrograde (cervico-fundal) or opposing contractions in the early luteal phase (Lyons *et al*., 1991; van Gestel *et al*., 2003). The frequency of sub-endometrial peristalsis has been shown to be higher in ovarian stimulation cycles compared with natural cycles but generally follows the same pattern as in natural cycles (Lesny *et al*., 1998; Zhu *et al*., 2012). Fanchin *et al*., showed that the pregnancy and implantation rates correlate negatively with the frequency of these contractions. Clinical , ongoing pregnancy and implantation rates decrease significantly in a stepwise manner with increasing frequency of uterine contractions at time of embryo transfer (Fanchin *et al*., 1998). The same findings has been reported in a small study in natural cycles (Jland *et al*., 1997). These findings from previous studies suggest that women with a background increase in the sub-endometrial peristalsis may have further increase in the frequency of contractions if embryo transfer is traumatic, decreasing the implantation potential. To decrease this detrimental impact of sub-endometrial hyper-peristalsis it is

imperative to exercise utmost care by performing gentle atraumatic embryo transfer aiming to minimize trauma to the endometrium and avoid touching the uterine fundus.

The practitioner's ability to perform atraumatic embryo transfer and other patient-related factors such as endometriosis (Bulletti *et al*., 2002) or the presence of fibroids (Yoshino *et al*., 2010) may also affect uterine contractions. It has, therefore, been suggested that using pharmacological agents that inhibit uterine contractions around the time of embryo transfer may lead to an improvement in implantation and pregnancy rates. The potential candidate agents to exert this effect include: oxytocin receptor antagonists (ORA); these drugs act by antagonising naturally circulating and locally synthesized oxytocin as well as vasopressin at their receptors in the myometrium promoting uterine relaxation (Pierzynski, 2011); prostaglandin synthetase (cyclooxygenase) inhibitors (PGSI); these drugs act by reducing levels of various prostaglandins such as PGF2 alpha, PGE2 and thromboxane A2, which are implicated in the induction of myometrial contractions in non-pregnant and pregnant uteri (Hagenfeldt, 1987; Olson *et al*., 2003; Marjoribanks *et al*., 2010); nitric oxide donors; these drugs are potent smooth muscle relaxants which act by increasing levels of nitric oxide and nitrites in the myometrium. This helps with vasodilatation and may help by inducing relaxation of the smooth muscles of the myometrium as shown in pregnancy (Lees *et al*., 1999; Bisits *et al*., 2004); beta-adrenergic receptor agonists; these drugs act by stimulating beta-adrenergic receptors in the myometrium, promoting the relaxation of the smooth muscle in cases of preterm labour (Leveno *et al*., 1986) and in the non-pregnant uterus (Fedorowicz *et al*., 2012); anti-cholinergic agents; these drugs act by antagonising the effect of acetylcholine at the muscarinic receptor promoting relaxation of the myometrial smooth muscles (Nakai *et al*., 2008; Kido *et al*., 2009); and calcium channel blockers; these drugs inhibit the influx of calcium ions through the cell membranes of smooth muscle, inhibiting

contractions. They have been used for tocolysis (Flenady *et al*., 2014) and treatment of dysmenorrhoea (Childress and Katz, 1994).

Many randomized controlled trials (RCTs) have been conducted to evaluate the efficacy of these drugs when administered in the peri-implantation period of assisted reproduction technique cycles (Shaker *et al*., 1993; Tsirigotis *et al*., 2000; Ohl *et al*., 2002; Pinheiro *et al*., 2003; Farzi, 2005; Bernabeu *et al*., 2006; Firouzabadi RD, 2007; Dal Prato and Borini, 2009; Moraloglu *et al*., 2010; Hanevik *et al*., 2012; Zargar, 2013; Ng *et al*., 2014). The results of these trials, however, have been conflicting. Consequently, their use in routine clinical practice within assisted reproduction treatment is not recommended. The aim of our study was to conduct a systematic review to identify all relevant RCTs and summarize the effect of these drugs on live birth and clinical pregnancy rates after assisted reproduction technique cycles as well as the safety of these medications.

<A>Materials and methods

The population of interest for our review were women undergoing assisted reproduction treatment cycles. The intervention of interest was administration of uterine relaxant drugs during the peri-implantation period of the assisted reproduction technique cycle. The peri-implantation period was defined as the period spanning from the day of oocyte retrieval until 3 days after embryo transfer in assisted reproduction technique cycles in humans, as this is the period when early events of the implantation process take place. The comparator intervention was placebo, no treatment or an alternative type of uterine relaxant drug. The primary outcome of interest was live birth rate. Secondary outcomes were clinical pregnancy rate, biochemical pregnancy rates, spontaneous abortion rates (including both biochemical

pregnancy losses and clinical spontaneous abortions), ectopic pregnancy rates and multiple pregnancy rates. We also sought to identify any reports of side-effects or serious reactions to the agents used in the included trials to assess any potential harm.

<**B**>Eligibility criteria

Studies were included if they were RCTs reporting on the use of any of the uterine relaxing agents in the peri-implantation period. Case reports, observational cohort studies, retrospective studies and non-randomized trials were excluded. Studies reporting only on surrogate outcomes (i.e. frequency of uterine contractions or patient discomfort) without reports on clinically relevant outcomes (i.e. clinical pregnancy rate, on-going pregnancy rate or live birth rate) were also excluded.

<**B**>Search and study selection

A literature search of multiple databases; *MEDLINE* (1978–2014), *Embase* (1978–2014), *Cochrane Register of Controlled Trials (CENTRAL)* (1978–014), ISI conference proceedings (1978–2014) and the controlled trials registers was conducted. Searches of the reference lists of the retrieved citations were conducted and contact made with the primary authors for other potentially relevant studies and to collect more information on the included studies. When only abstracts were available, the primary authors were also contacted for more information for analysis when feasible. No language restrictions were applied.

The search strategy (**Supplementary Table 1**) involved use of the MeSH and text words to form three groups of studies to retrieve RCTS reporting on uterine relaxants in assisted reproduction technique cycles using the following: (reproductive assisted techniques), (fertilization *in vitro*), (intracytoplasmic sperm injection) combined with Boolean operator (OR) and 'Randomised controlled trials' and 'controlled studies' combined with (OR) and

the various pharmacological agents/classes included in the review combined with (OR). The three groups of studies were combined together with the Boolean operator AND.

Two independent reviewers (MK and RD) selected the studies. The titles and abstracts were scrutinized, and the decision for inclusion or exclusion was made according to the predefined inclusion criteria. The two reviewers were not blinded to the full manuscript data. Any disagreements between the two reviewers were resolved by arbitration by a third reviewer (AC). The full manuscripts of the titles and abstracts that were considered to be relevant for inclusion were then obtained. In the case of duplicate publication, then the most recent and complete versions were selected. The full manuscripts were then examined and data were extracted by the same two independent reviewers.

<B>Validity assessment

Two reviewers (MK and RD) assessed the included manuscripts for validity using the quality criteria recommended by *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins, 2011). These included method and adequacy of randomization, achievement of allocation concealment (to assess selection bias), blinding of participants (to assess for performance bias) and assessors of outcomes (to assess for detection bias) and adequacy of follow-up (to assess for attrition bias) and reporting of all relevant outcomes (to assess for reporting bias). Assessment for publication bias in the included studies for the outcome of clinical pregnancy was made using assessment for the symmetry of the funnel Plot (**Supplementary Figure 1**) and Egger's test, and with the Bias statistic for the test 1.12 95% CI –0.7 to 2.96; $P = 0.2$ (Egger *et al*., 1997). Another assessment of publication bias for effect of small studies using Harbord modification of Egger's test showed bias statistic of 1.1 and 95% CI of –0.67 to 2.87 (Harbord *et al*., 2006) Both tests and the symmetry of the funnel plot indicating no evidence of publication bias. The same methodology for detecting

publication bias could not be carried out owing to the small number of studies reporting on live birth.

Meta-analysis of the results of the reported outcomes was carried out using Review Manager (RevMan version 5.3 for Windows) software from the Cochrane collaboration. Summary of effect size was presented using relative risk and random effects model. Assessment for statistical heterogeneity was made using I^2, with a value above 50% indicating substantial heterogeneity. The PRISMA group guideline for reporting of systematic reviews (Moher *et al.*, 2009) was used for compiling this systematic review.

It was decided *a priori* that, as the agents included in this review belong to different classes of pharmacological agents, it would not be appropriate to reach a practice recommendation from pooling findings of all the included studies in a single meta-analysis. Therefore, separate meta-analyses were conducted where feasible for each class of drugs.

To conclude and recommend from the appraised evidence, The Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group methodology for assessment of the strength of treatment effect and quality of evidence was used for all uterine relaxing agents and for individual groups of medications. This was done with the use of GRADE pro software available online from the GRADE working group (Guyatt *et al.*, 2008).

<A>Results

The literature search yielded a total of 2000 potentially relevant citations. After screening of titles and abstracts, and removal of duplicates, 1931 citations were excluded, as they clearly did not meet the inclusion criteria, and the full texts for 69 citations were assessed. Of these 69 articles, 52 were excluded as they did not satisfy our inclusion criteria (**Figure 1**). Of these 52 studies, four were non-relevant RCTs and three were RCTs reporting only on uterine contractions, 23 were prospective observational cohort studies, eight were retrospective

cohort studies, four were case reports, four were narrative reviews, three were animal studies, two were systematic reviews that were not relevant and one was a relevant systematic review but only available as poster presentation for use of oxytocin antagonists.

Seventeen studies were eligible for inclusion in our review. These studies included 3546 patients. The characteristics of the 17 included studies are presented in **Table 1**. The publication dates of the 17 studies that were included ranged between 1993 and 2014. Sample sizes varied from 27 to 800 participants. Two studies investigated the use of oxytocin receptor antagonists (atosiban) in the peri-implantation period (Moraloglu *et al*., 2010; Ng *et al*., 2014). Four studies investigated the use of beta adrenergic receptors agonists (Tsirigottis *et al*., 2000; Pinheiro *et al*., 2003; Rabiee *et al*., 2011; Hanevik *et al*., 2012). Seven studies investigated the use of prostaglandin synthetase inhibitors (Moon *et al*., 2004; Bernabeu *et al*., 2006; Asgharnia *et al*., 2007; Firouzabadi *et al*., 2007; Dal Prato *et al*., 2009; Sohrabvand *et al*., 2009; Sohrabvand, 2014). Three studies reported the findings of trials investigating the use of the nitric oxide donor in the form of nitroglycerin patch (Shaker *et al*., 1993; Ohl *et al*., 2002; Farzi *et al*., 2005) and two studies investigated the use of the antimuscarinic agent Hyoscine (Sohrabvand *et al*., 2009; Zargar, 2013).

The search did not yield any relevant studies reporting on the use of calcium channel blockers; however, a registered trial for the use of nifedipine in the peri-implantation period in frozen embryo transfer cycles was identified. This study has not yet started recruiting (Meir, 2014).

The results of the Cochrane quality assessment for the included trials are presented in **Table 2**, **Figure 2** and **Supplementary Tables 2–5**. The quality of the included studies was generally low. The methods for randomization were unclear or inadequate in 11 out of the 17 included trials. Allocation concealment was inadequate or unclear in 13 trials. Participant

blinding was lacking or unclear in nine trials. Assessor blinding was lacking or inadequately described in 13 trials. In four of the 17 studies, follow-up was incomplete or a large number of participants dropped out after recruitment, and reporting of clinically relevant outcomes was incomplete in 15 out of the 17 trials.

<**B**>Live birth rate

Only four studies (totalling 1317 patients) reported on the outcome of live birth. One study compared oxytocin receptor antagonist atosiban with no treatment (Ng *et al*., 2014). This study did not show any statistically significant effect on live birth rate, with relative risk of 1.05 (95% CI 0.87 to 1.25). One study (Hanevik *et al*., 2012) compared beta adrenergic receptor agonist versus no treatment. This study found no significant effect with a relative risk of 0.85 (95% CI 0.60 to 1.20) for live birth. Two studies compared nitric oxide donor with no treatment (Ohl *et al*., 2002; Farzi *et al*., 2005), and showed a relative risk of 1.11 (95% CI 0.68 to 1.80) for live birth showing a non-significant effect (**Figure 3**).

<**B**>Clinical pregnancy rate

Thirteen studies reported on the outcome of clinical pregnancy rate with a total sample size of 2760 patients. Only one study reporting on the use of the anticholinergic agent Hyoscine (Zargar *et al.*, 2013) showed a significant beneficial effect. This study gave a relative risk of 2.33 (95% CI 1.15 to 4.74). This was the only study reporting on clinical pregnancy outcome after the use of anticholinergic drugs, and included only small number of patients. Two studies reported on the use of ORA (Moraloglu *et al*., 2010; Ng *et al*., 2014). Meta-analysis of these two studies gave a pooled relative risk of 1.26 (95% CI 0.85 to 1.87) with no detectable statistically significant effect (**Figure 4**). Four studies investigated the use of

Comment [S1]: Author: it is usual to use generic drug names. should the generic be used here (hyoscine butylbromide)?

PGSI (Moon *et al*., 2004; Bernabeu *et al*. 2006; Firouzabadi *et al*., 2007; Dal Prato *et al*. 2009). Pooling of these four studies gave a relative risk of 1.36 (95% CI 0.87 to 2.11), with no statistically significant effect. Statistically significant heterogeneity was found in the two meta-analyses for oxytocin receptor antagonists (I^2 = 73%; *P* = 0.05) prostaglandins synthetase inhibitors (I^2 = 77%; *P* = 0.004).

Other classes of uterine relaxing agents also did not show statistically significant effect in clinical pregnancy rate compared with placebo. Three studies (Tsirigottis *et al*., 2000; Pinheiro *et al*., 2003; Hanevik *et al*., 2012) reported on effect of beta adrenergic receptor agonist on clinical pregnancy rate. Pooling of the findings of these studies showed a relative risk of 0.99 (95% CI 0.59 to 1.66) (**Figure 4**). Three studies (Shaker *et al*.,1993; Ohl *et al*., 2002; Farzi *et al*., 2005) compared nitric oxide donors with no treatment with pooled relative risk of 0.97 (95% CI 0.70 to 1.36) of these results showing no significant effect on clinical pregnancy rate (**Figure 4**).

Publication bias, as displayed by the symmetrical funnel plot as shown in (**Supplementary Figure 1**) and Egger test result above, was not evident.

<B>Other secondary outcomes

Ten studies reported on the outcome of biochemical pregnancy rate, with one study (Sohrabvand *et al*. 2009) reporting on more than one drug (**Supplementary Figure 3**). No significant effect was found on biochemical pregnancy rate for any of the groups of uterine relaxants with RR 1.10 (95% CI 0.96 to 1.25), 0.93 (95% CI 0.69 to 1.26), 0.88 (95% CI 0.34 to 2.23), 1.07 (95% CI 0.74 to 1.55) and 1.71 (95% CI 0.92 to 3.19) for the ORA (Ng *et al*., 2014), PGSI (Bernabeu *et al*., 2006; Firouzabadi *et al*., 2007; Dal Prato *et al*., 2009; Sohrabavand *et al*., 2009; Sohrabavand et *al*., 2014) beta-adrenergic receptor agonists

(Rabiee *et al*., 2011), nitric oxide donors (Ohl *et al*., 2002; Farzi *et al*., 2005) and antimuscarinic agents (Sohrabavand *et al*., 2009; Zargar *et al*., 2013), respectively.

Nine studies reported on the spontaneous abortion rate (**Supplementary Figure 4**). The meta-analyses on spontaneous abortion rates showed no statistically significant difference between the intervention and control groups in any of the uterine relaxants groups with RR 0.95 (95% CI 0.64 to 1.40), 0.57 (95% CI 0.27 to 1.22), 0.60 (95% CI 0.15 to 2.42) and 0.89 (95% CI 0.40 to 1.99) for ORA (Moraloglu *et al*. 2010; Ng *et al*., 2014), PGSI (Bernabeu *et al*., 2006; Firouzabadi *et al*., 2007; Dal Prato *et al*., 2009), B adrenergic receptor agonists (Pinheiro *et al*., 2003; Hanevik *et al*., 2012) and nitric oxide donors (Ohl *et al*., 2002; Farzi *et al*., 2005), respectively.

Only three studies reported on the outcome of ectopic pregnancy; one study reporting on oxytocin antagonist (Ng *et al*., 2014), one on prostaglandin synthetase inhibitors (Dal Prato *et al*.,2009) and one study on nitric oxide donors (Ohl *et al.*, 2002). No statistically significant difference was found in the incidence of ectopic pregnancy in any of these individual studies between those that received uterine relaxing agents compared with no treatment with RR and 95 % CI of 0.82 (0.34 to 1.98), 0.95 (0.06 to 14.13) and 0.56 (0.05 to 5.89) for the three studies, respectively.

Five studies reported on the outcome of multiple pregnancy; two studies on the use of oxytocin receptor anatgonists (Moraloglu *et al*., 2010; Ng *et al*., 2014), two studies on the use of prostaglandin synthetase inhibitors (Bernabeu *et al*., 2006; Firouzabadi *et al*., 2007) and one on the use of nitric oxide donors (Ohl *et al*., 2002) (**Supplementary Figure 5**). The pooled RR and corresponding 95% CI were 0.86 (0.66 to 1.13) and 1.06 (0.57 to 1.97) for the ORA and PGSI respectively (**Supplementary Figure 5**). The RR and 95% CI for the study on nitric oxide donor was 0.95 (0.22 to 4.14).

No serious side-effects were reported in any of the trials included in the review (**Table 1**). The study by Ng *et al*., (2014), which used the oxytocin antagonist atosiban reported a higher rate of congenital abnormalities in the treatment group (3.2% compared with 1.6% in the placebo group).

Using the GRADE approach for appraising the quality of included studies and effect size reported on the meta-analyses of various groups of uterine relaxants, we have shown that the current evidence for all groups showed non -significant effect based on very weak evidence (**Table 3** and **Supplementary Tables 2–5**) (Guyatt *et al*., 2008).

<A>Discussion

This systematic review sought to summarize the current evidence for the use of different classes of uterine relaxants at the peri-implantation period in during assisted reproduction. Overall, the systematic review and meta-analyses shows that the identified evidence does not support the routine clinical use of these agents in assisted reproduction treatment cycles. Rigorous RCTs still need to be conducted, as the quality of the included studies was generally low with significant clinical and statistical heterogeneity in most meta-analyses.

<B>Oxytocin receptor antagonists

In the meta-analyses for the ORA, although there was a no statistically significant effect on live birth and clinical pregnancy rates, the number of patients included in the trials on ORA was 980 patients, which is underpowered to identify significant difference in live birth or clinical pregnancy rate at the set statistical significance limits. Thirty more clinical pregnancies occurred in the arm using oxytocin antagonist compared with placebo or no treatment. Although the 95% confidence interval for the clinical pregnancy outcome was not

statistically significant, the pooled point estimate of the risk ratios for clinical pregnancy rates was 1.26.

The oxytocin receptor antagonist used in the trials in this review was atosiban, a mixed vasopressin and oxytocin antagonist and its use was limited to 2.5 h at the time of embryo transfer. Whether the use of a more selective oxytocin receptor antagonist such as barusiban, a longer duration of use in the peri-implantation period, or both, might show a greater beneficial effect remains to be seen. The role of ORA in patients with recurrent implantation failure is still unclear. It has been shown in an observational study that ORA are beneficial in cases of recurrent implantation failure where cryopreserved embryos were transferred in a hormonal replacement cycles (Lan *et al*., 2012). The trial by Ng *et al*. (2014) did not report a statistical increase in clinical pregnancy rate or live birth rate in patients with repeated cycles, although the authors cautioned against extrapolating this to patients with recurrent implantation failure. The result of an RCT registered in the Clinicaltrials.gov recruiting for the use of ORA in patients with repeated implantation failure is awaited (NCT01673399) (Decleer, 2015).

The disadvantage of oxytocin receptor antagonists is the financial cost. It also requires intravenous infusion and carries the potential for serious side-effects. These include pulmonary oedema, which has been reported in obstetric populations using atosiban for tocolysis (Donders *et al*., 2008; Fernandez *et al*., 2011). In this review, no serious side-effects of atosiban or other classes of the drugs were reported.

<**B**>Oral and transdermal uterine relaxants

The use of oral or transdermal agents is generally more appealing to patients and clinicians. In this review, the only oral agent that showed a significant effect on clinical pregnancy was the anticholinergic agent, hyoscine. This was, however, based on single clinical trial, which included a relatively small number of patients with low quality assessment grading and no reporting on the primary outcome of live birth rate (Zargar, 2013). Another small trial showed improvement of biochemical pregnancy rate with hyoscine but no clear data on clinical pregnancy rate (Sohrabvand *et al*., 2009). Therefore, caution must be taken when drawing any definitive conclusions for the use of these agents in routine clinical practice.

No statistically significant effect was found on live birth or clinical pregnancy from meta-analyses of other oral or transdermal agents. In the only RCT that exclusively included patients with recurrent implantation failure, however, a statistically significant effect was reported on clinical and ongoing pregnancy rate in the treatment arm using ritodrine (Tsirigotis *et al*., 2000).

Interestingly we did not identify any studies that investigated the effectiveness of calcium channel blockers. A RCT has been egistered in clinicaltrials.gov (NCT02072291) that has not started recruiting yet and will investigate the use of nifedipine on uterine contractility and pregnancy rate in frozen embryo replacement cycle (Meir, 2014). The calcium channel blockers might be a suitable alternative oral agent to ORA as the voltage and ligand gated calcium channels are more ubiquitous structures in the smooth muscle membranes of the myometrium (Sanborn, 2000) compared with the oxytocin receptors, with ORA exerting their actions mainly through paracrine factors including prostaglandins rendering them functionally redundant especially in the non-pregnant uterus (Ivell *et al*., 2001)

Reassuringly, our review showed no increase in the incidence of adverse fertility outcomes. The relative risks for spontaneous abortion, ectopic pregnancy rate and multiple pregnancy rate were not significantly different between the treatment and control groups. No major side-effects were reported from any of the agents studied in this review. Although a higher rate of congenital abnormalities was reported in the ORA treatment arm in one of the trials compared with placebo, the number reported was too small to allow conclusions to be drawn. Furthermore, the rate of congenital abnormalities reported was not markedly different from the generally expected congenital abnormalities in pregnant women, and the ORA was used for a short period before the actual implantation would have occurred, casting further doubt on a cause and effect relationship.

<**B**>Strengths and weaknesses

The strengths of this review mainly lie in its extensive and rigorous search strategy. Systematic searches were conducted on multiple healthcare literature databases without language restriction. There was also no evidence of publication bias. The main limitation of this review is the low quality of the included studies. Most studies were of relatively small sample size to enable the authors to detect a clinically important minimal difference in live birth rate and other important outcome. The included studies were also not statistically heterogeneous, which would limit the interpretation of our findings. The significant heterogeneity detected in these trials can be partially explained by different inclusion and exclusion criteria among included trials with differences in dose, duration and type of uterine relaxants used. Most of the trials had one or more of element of bias as shown in the quality assessment tables (**Tables 2, 3** and **Supplementary Tables 2–5**). This tends to overestimate the treatment effect limiting confidence in the findings. This has been reflected in the very low grading of the quality of evidence from the included trials, as appraised using the

GRADE approach, owing to serious methodological shortfalls, e.g lack of allocation concealment or blinding.

Subgroup analyses could not be carried out owing to lack of available data reporting exclusively on certain groups of patients, e.g recurrent implantation failure, endometriosis. Sensitivity analyses and meta-regression to adjust for effect of confounding variables was not deemed necessary, as most analyses did not show statistically significant effects.

Another important weakness in the included studies was the general lack of power to detect minimally important difference in primary outcome even after pooling studies within groups, e.g. the predicted sample size required to detect a minimally significant effect of 5% improvement in live birth assuming a background rate of 35% will be nearly 2942 patients (1471 patients in each group) for a study with a power of 0.8 and statistical significance set at 0.05. This sample size was not achieved in any of the included meta-analyses indicating possibility of false negative findings (Type II error).

In conclusion, insufficient evidence is currently avaailble to support the use of uterine relaxant agents around the time of embryo transfer in routine clinical practice. Given the methodological short comings of the current evidence, however, larger robust RCTs are clearly needed to investigate the usefulness of these agents if any (in particular, anticholinergic agents, oxytocin receptor antagonists and calcium channel blockers). It is possible that careful patient selection for the use of these agents might show greater benefit in future RCTs, e.g. patients who reported pain after previous embryo transfer, those where difficult embryo transfer is anticipated or patients with adenomyosis/endometriosis. With better scanning techniques and equipment, uterine hyper-peristalsis might be more readily detectable. This would allow the improved identification of patients who might benefit from these agents.

Declaration

The authors report no financial or commercial conflict of interest.

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Supplementary Figure legends

Supplementary Figure 1: Funnel plot for studies reporting on clinical pregnancy rate after the use of uterine relaxants in the peri-implantation period.

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Supplementary Figure 2: Quality parameters of the included studies.

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Supplementary Figure 3: Forest plot of the risk ratios of biochemical pregnancy rate after use of uterine relaxants at the peri-implantation period.

Supplementary Figure 4: Forest plot of the risk ratios of spontaneous abortion rate after use of uterine relaxants at the peri-implantation period.

Supplementary Figure 5: Forest plot of the risk ratios of multiple pregnancy rate after use of uterine relaxants at the peri-implantation period.

Figure legends

Figure 1: Selection process for included papers. RCT, randomized controlled trial.

Figure 2: Quality assessment for sources of bias in the included studies.

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Figure 3: Forest plot of the meta-analysis of risk ratio of the effect of uterine relaxants (nitric oxide donors) used in the peri-implantation period on the live birth rate.

Figure 4: Forest plot of the risk ratios of clinical pregnancy rate after use of uterine relaxants at the peri-implantation period.

Figure 1

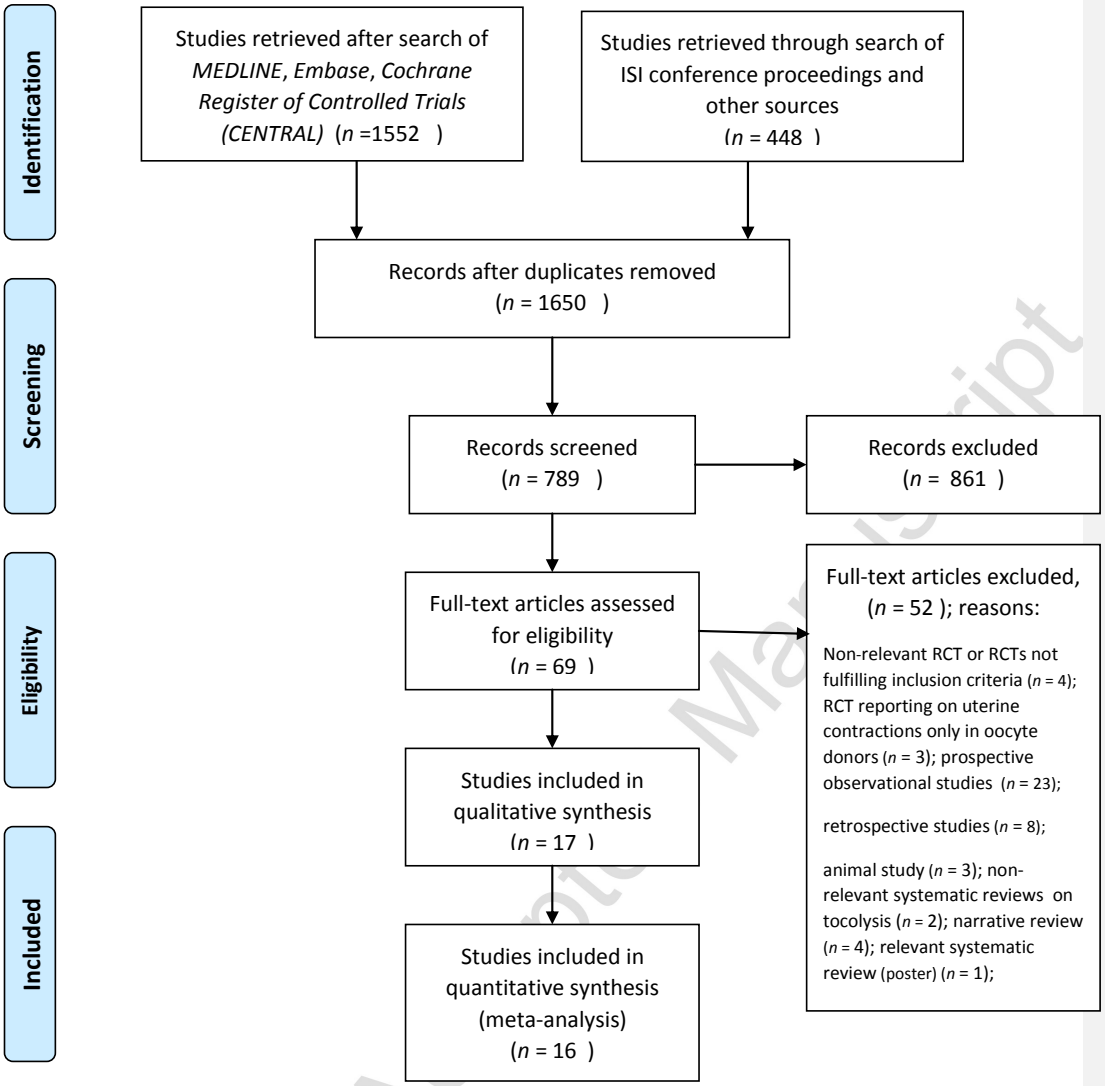

Figure 2

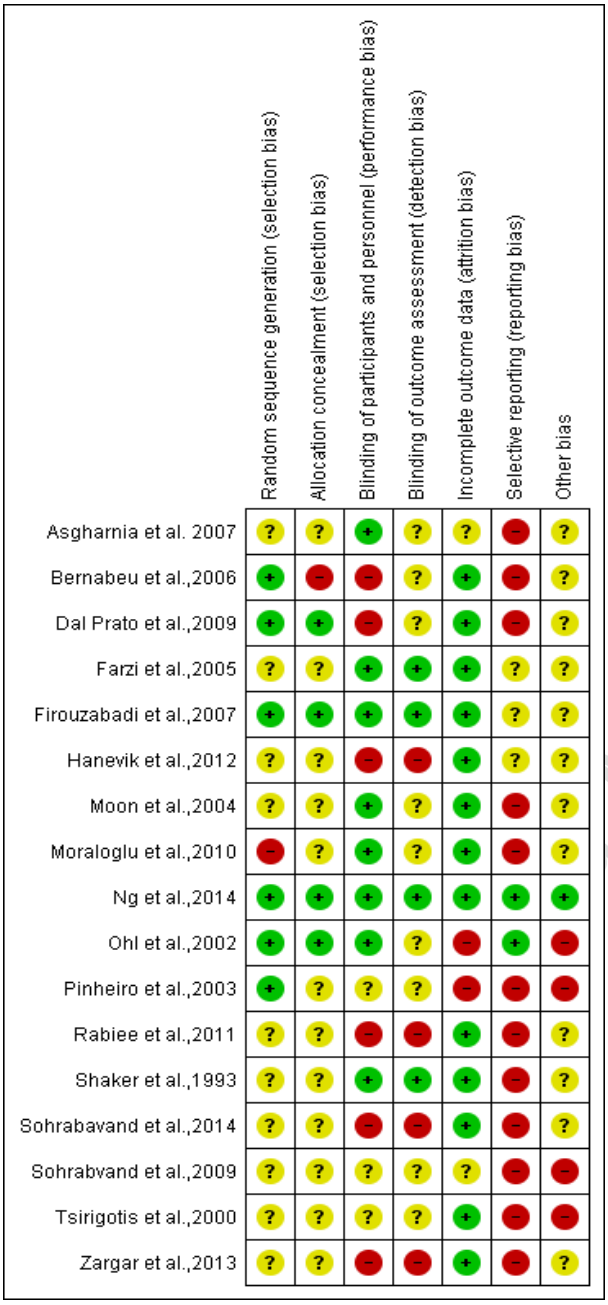

Figure 3

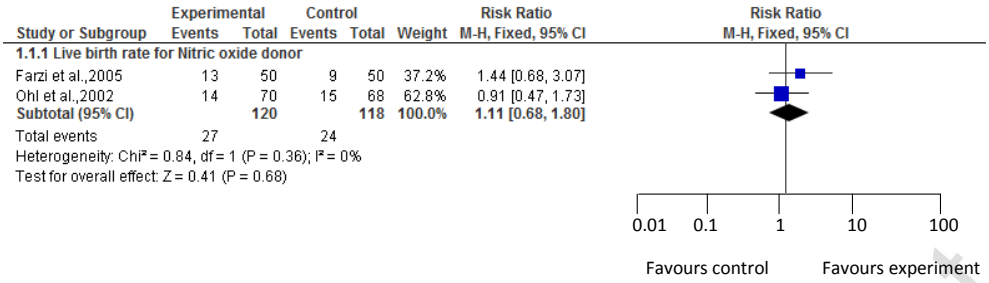

Figure 4

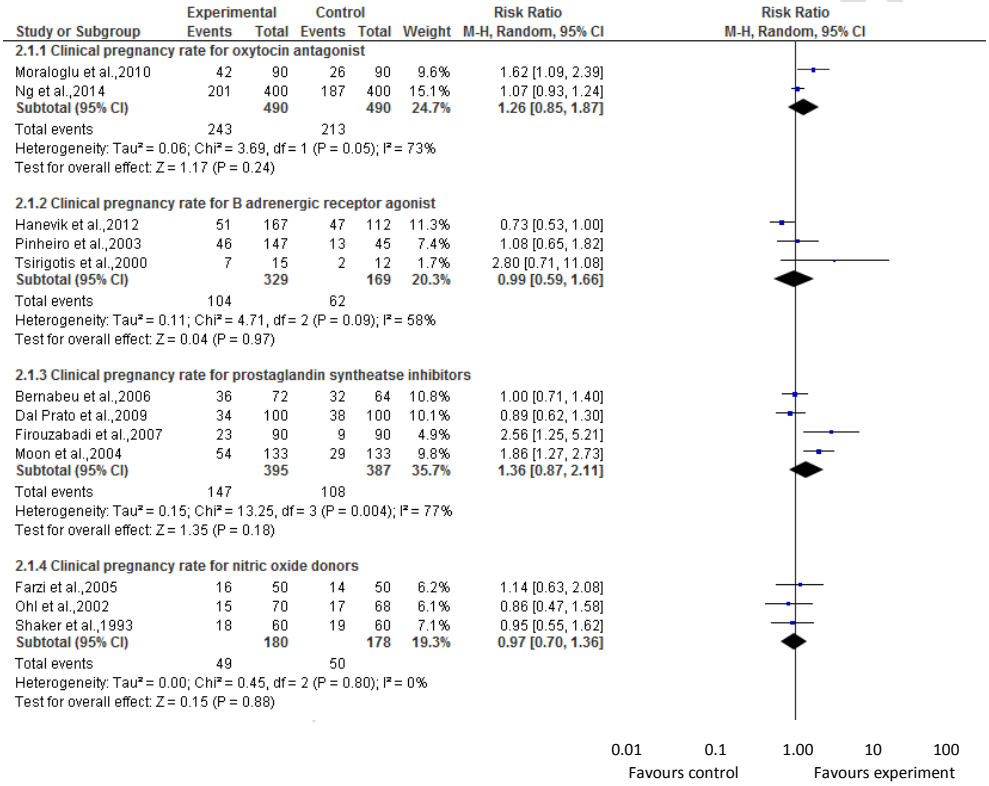

Table 1. Characteristics of the included studies.

Study	*Participants*	*Intervention*	*Comparison (control)*	*Outcomes*	*Results* *(treatment versus control group)*
Asgharnia *et al*., 2007	500 patients undergoing IVF–ICSI treatment because of male factor, tubal, anovulatory and unexplained factor infertility. Women with uterine pathology, such as fibroids or Asherman's syndrome, were excluded.	Intervention group (*n* = 250) received long protocol and ovarian stimulation with piroxicam 10 mg orally 1–2 h before embryo transfer.	Control group (*n* = 250) received same long protocol and ovarian stimulation and placebo tablet 1–2 h before embryo transfer.	Clinical pregnancy rate	NA[a]
Bernabeu *et al*., 2006	173 first cycle oocyte recipients following IVF–ICSI with no allergies to non-steroidal anti-inflammatory drugs and no history of neurological or gastrointestinal diseases. Women who had experienced recurrent spontaneous abortion or severe endometriosis and endometrial pathology were excluded.	Egg donation treatment protocol using ethinyl oestradiol and vaginal micronized progesterone and Indomethacin 100 mg given as rectal suppositories every 12 h (three doses) starting on the night before embryo transfer. The non-intervention group did not receive any placebo.	Standard egg donation treatment for the control arm without placebo treatment.	Biochemical pregnancy rate Clinical pregnancy rate Implantation rate Spontaneous abortion rate	43/72 (59.7%) versus 40/64 (62.5%) 36/72 (50%) versus 32/64 (50%) 52/191 (27.2%) versus 44/168 (26.2%) 7/43 (16.2%) versus 8/40 (20%) 13/36 (36.1%)

				Multiple pregnancy rate	versus11/32 (34.4%)
Dal Prato *et al*., 2009	200 women younger than 44 years at enrolment for IVF–ICSI cycles for male factor, tubal or endometriosis-related infertility with no more than two previous embryo transfers. Patients with inadequate ovarian reserve as indicated by high baseline FSH over15 IU/l or previous poor response or patients with more than two embryo transfers were excluded.	Standard IVF–ICSI treatment with piroxicam 10 mg orally 1–2 h before embryo transfer.	Standard IVF–ICSI treatment without placebo.	Biochemical pregnancy rate Clinical pregnancy rate Implantation rate Spontaneous abortion rate Ectopic pregnancy rate	37/100 (37%) versus 47/100 (47%) 34/100 (34%) versus 38/100 (38%) 48/250 (19.2%) versus 56/256 (21.9%) 7/37 (18.9) versus 14/47 (29.8.%) 1/34 (2.9%) versus 2/38 (5.3%)
Farzi *et al*., 2005	100 consecutive patients (cycles) undergoing IVF–ICSI treatment in standard long protocol for pituitary down regulation and ovarian stimulation for various male, female and combined indications	A single oral dose of nitrogylcerin (0.4 mg 15 min before embryo transfer).	A single oral dose of placebo 15 min before embryo transfer.	Biochemical pregnancy rate Clinical pregnancy rate	19/50 (38%) versus 17/50 (34%) 16/50 (32%) versus 14/50 (28%)

	Patients with hypotension or hypertension or ASA grade greater than 1 or marked variation of supine and standing blood pressure were excluded.			Live birth rate Spontaneous abortion rate	13/50(26%) versus 9/50 (18%) 6/19 (31.6%) versus 8/17 (47%) Three biochemical pregnancy losses and tree clinical spontaneous abortions in the treatment group and three biochemical pregnancy losses and five clinical spontaneous abortions in the control group
Firouzabadi *et al*., 2007	180 patients (cycles) undergoing IVF treatment for tubal, ovarian, male mixed and unexplained infertility all having standard long protocol of pituitary down regulation and ovarian stimulation.	Single oral dose of piroxicam 10 mg administered to 90 patients (cycles) 1–2 h before embryo transfer.	Placebo treatment administered orally to patients 1–2 h before the embryo transfer.	Implantation rate Biochemical pregnancy rate Clinical pregnancy rate Spontaneous	12.3% versus 7.7% 24/90 (26.7)% versus 14/90 (15.6%) 23/90 (25.6%)

				abortion rate Multiple pregnancy rate	versus 9/90 (10.0%) 1/24 (4.2%) versus 5/14 (35.7%) 2/24 (8.3%) versus 1/14 (7.1%)
Hanevik *et al*., 2012	279 patients younger than 40 years with no abnormal uterine anatomy or azoospermia, who were non-diabetic with no hyperthyroidism and normal baseline FSH and normal quality embryos on day 2 after oocyte retrieval	Standard IVF–ICSI treatment with low-dose aspirin 75 mg/d from embryo transfer until pregnancy test and up to 9 weeks gestation if patient is pregnant. Patients in the intervention group also received three doses of terbutaline 5 mg 3 h before embryo transfer, 6 h after embryo transfer and at 8 am the morning after embryo transfer.	Standard IVF–ICSI without placebo treatment.	Live birth rate Clinical pregnancy rate Spontaneous abortion rate Cancellation rate	48/167 (28.7%) versus 38/112 (33.9%) 51/167 (30.5%) versus 47/112 (42.0%) 3/51(5.9%) versus 9/47 (19.1%) 14/167(8.4%) versus 6/112 (5.4%)
Moon *et al*., 2004	266 patients undergoing IVF–ICSI (188 fresh cycles and 78 frozen thawed embryo transfer) treatment	Standard IVF–ICSI treatment with piroxicam 10 mg orally 1–2 h before embryo transfer.	Standard IVF–ICSI fresh cycles or frozen thawed embryo transfer	Implantation rate Clinical pregnancy rate	18.7% versus 8.6% 54/133 (40.6%) versus 29/133

	for male factor endometriosis, unexplained endometriosis and tubal factors infertility. No clear inclusion or exclusion criteria.		protocol with placebo at the time of embryo transfer.		(21.8%)
Moraloglu *et al*., 2010	180 patients having fresh IVF–ICSI treatment for male factor, tubal factor and unexplained infertility Patients were included if basal FSH was less than10 IU/L, having first IVF cycle, long protocol and have at least two top quality embryos. Exclusion criteria were female age over 39 years, endocrine disorders (such as thyroid disorders and premature ovarian failure) severe male factor infertility, endometriosis, patients with more than three previous failed IVF cycles, frozen thawed embryo transfer, uterine anomalies, fibroids, hydrosalpinges and	Standard IVF–ICSI treatment with long protocol and intravenous infusion of atosiban at a bolus dose of 6.7 5mg 30 min before embryo transfer, continued at a rate of 18 mg/h then at a rate of 6mg/h for 2 h after embryo transfer (total administered dose 37.5 mg).	Standard IVF–ICSI fresh cycles in long protocol with intravenous infusion of identical medicated bags with saline.	Implantation rate Clinical pregnancy rate Spontaneous abortion rate Multiple pregnancy rate	57/279 (20.4%) versus 34/270 (12.6%) 42/90 (46.7%) versus 26/90 (28.9%) 7/42 (16.7%) versus 6/26 (23.1%) 6/42 (14.3%) versus 5/26 (19.2%)

	patients who have a history of difficult embryo transfer.				
Ng *et al*., 2014	800 patients having fresh IVF–ICSI treatment were recruited if they were younger than 43 years and had normal uterine cavity on ultrasound.	Standard IVF–ICSI treatment and at fresh embryo transfer - intravenous infusion of atosiban at a bolus dose of 6.75 mg 30 min before embryo transfer, continued at a rate of 18 mg/h then at a rate of 6 mg/h for 2 h after embryo transfer (total administered dose 37.5 mg)	Standard IVF–ICSI treatment and at fresh embryo transfer intravenous infusion of identically medicated bags with normal saline.	Live birth rate Ongoing pregnancy rate Clinical pregnancy rate Biochemical pregnancy rate Spontaneous abortion rate Multiple pregnancy rate	159/400 (39.8%) versus 152/400 (38.0%) 171/400 (42.8%) versus 153/400(38.3%) 201/400 (50.3%) versus 187/400 (46.8%) 217/400 (54.3%) versus 198/400 (49.5%) 37/217 (17.1%) versus 35/198 (17.7%) 65/217 (30.0%) versus 68/198 (34.3%)

				Ectopic pregnancy rate Side-effects Congenital abnormalities	9/217 (4.1%) versus 10/198 (5.1%) No serious adverse events 4/125 (3.2%) versus 2/125 (1.6%)*
Ohl *et al*., 2002	138 patients with a history of at least two previous failed IVF–ICSI cycles despite transfer of two good quality embryos in each cycle. Patients were excluded if they did not have two good quality embryos to transfer during the trial or if they had a history of hypersensitivity to nitric oxide donors, heart failure, severe anaemia, high intracranial blood pressure or high intraocular pressure.	IVF–ICSI treatment with long protocol and ovarian stimulation and 5 mg nitroglycerin patch applied once daily on the morning before embryo transfer and removed at bedtime. The nitroglycerin patch was then continued until either the results of pregnancy test was available or menstruation starts.	IVF–ICSI treatment with long protocol and ovarian stimulation and placebo patch starting on the day before embryo transfer and continued till day of pregnancy test. Micronized progesterone 200 mg intra-vaginally twice daily was used as luteal phase support in both intervention and control arms.	Uterine artery pulsatility index on the day before and after embryo transfer Implantation rate Biochemical pregnancy rate Clinical pregnancy rate Live birth rate	 10.6% versus 11.6% 20/70 (28.6%) versus (19/68) (27.9%) 15/70 (21.4%) versus 17/68 (25%)

				Spontaneous abortion rate	14/70 (20%) versus 15/ 68 (22.1%)
				Ectopic pregnancy rateMultiple pregnancy rate	5/20 (25%) versus 3/19 (15.8%)
				Side effects	1/20 (5.0%) versus 1/19 (5.3%) 3/20 (15.0%) versus 3/19 (15.6%) One patient in the placebo group had nervousness, insomnia, constipation and feeling of weakness

Pinheiro *et al*., 2003	225 patients undergoing ICSI treatment for male factor infertility.	Long protocol for ovarian stimulation using fixed dose of 300 IU/day and two intervention arms: Group 1, *n* = 90 patients, taking terbutaline 10 mg orally daily for 15 days starting on the day of oocyte retrieval. Group 2, *n* = 90 patients taking ritodrine 20 mg orally daily for the same period as Group 1).	Group 3, *n* = 45 (control arm) received no treatment apart from the long protocol for ovarian stimulation and Uterogestan 800 mg intravaginally daily until day of pregnancy test. Up to three embryos were transferred in all arms of the trial.	Implantation rate Clinical Pregnancy rate Spontaneous abortion rate	61/409 (14.9%) versus 19/126 (15.1%) 46/147 (31.3%) versus 13/45 (28.9%) 9/46 (19.6%) versus 2/13 (15.3%)
Rabiee *et al*., 2011	100 women aged 25–35 years with infertility for over 5 years undergoing IVF–ICSI treatment for male factor, tubal factor, endometriosis or unexplained infertility. Patients were eligible for inclusion if they had a morphologically normal uterus. Exclusion criteria were any systemic illnesses.	Intervention group (*n* = 50) received the same protocol in the control arm, as well as ritodrine 10 mg orally twice daily for 10 days starting from the day after oocyte retrieval.	Control group (*n* = 50) received long protocol of ovarian stimulation, Acetyl salicylic acid 80 mg/d, erythromycin 400 mg orally four times a day, heparin 5000 IU twice daily and progesterone 100 mg IM and no placebo.	Biochemical pregnancy rate Side-effect	7/50(14.0%) versus 8/50 (16.0%) One patient in the ritodrine group had headache and vertigo

Sohrabvand *et al*., 2014	50 patients aged 20–35 years undergoing IVF–ICSI treatment for tubal factor or ovulation disorder or severe male factor infertility with standard long protocol for pituitary down regulation and ovarian stimulation Exclusion criteria were patients with systemic diseases and endometriosis	Single oral dose of Piroxicam 10 mg 30 min before embryo transfer with bed rest for 30 min after embryo transfer.	No treatment before embryo transfer and bed rest for 30 min after embryo transfer.	Biochemical pregnancy rate Abdominal cramps	8/25 (32.0%)vs 9/25 (36.0%) No patients in treatment groups had cramps and four patients in the control group had abdominal cramps
Sohrabvand *et al*., 2009	66 women recruited for three arms randomized controlled trial. Patients were included if they had tubal factor, severe male factor or ovulatory infertility and they were having their first assisted reproduction treatment cycle. Exclusion criteria were patients with endometriosis and patients with systemic illnesses.	Two interventions groups: Group A (*n* = 22) had in addition to long ovarian stimulation protocol, standard IVF–ICSI received 10 mg rectal suppository of indomethacin 30 min before embryo transfer. Group B (*n* = 22) had the same protocol of ovarian stimulation and IVF, and received hyoscine 100 mg rectal suppository.	Control group (*n* = 22) received same protocol of ovarian stimulation and IVF–ICSI without any additional placebo treatment. Patients in all groups had a bed rest for 30 min after embryo transfer.	Biochemical pregnancy rate Side-effects	10/22 (45.5%) for hyoscine and 3/22 (13.6%) for indomethacin versus 8/22 (36.4%) No patients in the active treatment groups had side effects and three in the control group had abdominal cramps.

Tsirigotis *et al*., 2000	27 Patients with three or more previous failed IVF attempts who also had lower abdominal pain after embryo transfer for few days or until pregnancy test. Patients had standard IVF and embryo transfer treatment in both treatment and control groups	Intervention group received oral ritodrine 5 mg /6 h from the day of oocyte collection up to the day of pregnancy test.	Control group did not receive any placebo	Clinical pregnancy rate Ongoing pregnancy rate	7/15(46.7%) versus 2/12 (16.7%) 5/15(33.3%) versus 0/12 (0%)
Shaker *et al*., 1993	120 patients having IVF and embryo transfer were included.	Intervention group had ovarian stimulation and IVF and received sublingual glyceryl trinitrate at the time of embryo transfer.	Control group had the same protocol of ovarian stimulation and IVF and received a placebo sublingual tablet at the time of embryo transfer.	Clinical pregnancy rate	18/60 (30.0%) versus 19/60 (31.7%)
Zargar *et al*., 2013	142 patients aged 21–41 years undergoing IVF–ICSI cycle for the treatment of male factor, tubal factor or ovulatory dysfunction and unexplained infertility. Patients had long protocol for pituitary down regulation and ovarian stimulation.	An injection of hyoscine (25 mg) 30 min before embryo transfer.	No injections given to the control group.	Biochemical pregnancy rates Clinical pregnancy rate	21/71 (29.6%) versus 9/71 (12.7%) 21/71 (29.6%) versus 9/71(12.7%)

[a]No numerical or percentage data available despite attempt of contacting the primary author.

ASA, American Society of Anesthesiologists; ICSI, intracytoplasmic sperm injection.

Table 2. Quality assessment of the included studies.

Study	*Randomization*	*Allocation Concealment*	*Blinding of participants*	*Blinding of assessors*	*Adequacy of reporting*	*Selective reporting*	*Other biases*
Asgharnia *et al*., 2007	Unclear method of Randomization.	Unclear if allocation concealment was achieved.	Placebo was used so blinding was likely achieved.	Unclear if assessors were blinded to allocation of patients.	Unclear if all patients included are reported.	Only statistical significance between pregnancy outcomes reported and no data suitable for analysis on and no data on live birth and other important outcomes.	Unclear if patients only allowed one cycle of treatment.
Bernabeu *et al*., 2006	Adequate; random sequence numbers generated by epidemiologist.	Inadequate.	No; controls did not receive placebo.	Unclear.	Incomplete; around 21% of patients recruited were not reported on as they did not complete their treatment.	Only clinical pregnancy and implantation rates and multiple pregnancies and spontaneous abortion rates; no data on live	

						birth or ectopic pregnancy rates or side-effects.	
Dal Prato *et al*., 2009	Adequate; independent statistician.	Adequate. third party and using dark sealed envelopes.	No; control arm did not receive placebo.	Unclear.	Adequate.	Clinical pregnancy, implantation and spontaneous abortion rates reported; no reporting on live birth rates, multiple pregnancy rate or side-effects.	Unclear if results are biased by protocol of study as only Three oocytes were inseminated in each patient.
Farzi *et al*., 2005	Unclear method of randomization.	Unclear	Yes; by use of placebo	Yes .	Adequate reporting on all patients included in the study	No reporting on ectopic pregnancy or multiple pregnancy rate or side-effects.	
Firouzabadi *et al*., 2007	Computer-generated random table.	Adequate by a computer-generated randomization and blinding of staff.	Yes; by use of placebo.	Yes.	Adequate reporting of all patients included in the study.	No reporting on live birth rate and ectopic pregnancy rate or side-effects of the drug but unlikely to change conclusion.	
Hanevik *et al*.,	Inadequate;	Inadequate;	No; patients had to pay	No.	Adequate follow-up and reporting	No reporting on adverse	

2012	randomized blocks of 10 envelopes with 3:2 ratio for intervention/control picked randomly by patients.	sealed envelopes used from blocks of 10 with (potential of knowing allocation in advance by care provider and patient).	partly for adjuvant treatment and no placebo was used.		on participants.	outcomes or side-effects of medications.	
Moon *et al*., 2004	Unclear method of randomization.	Unclear.	Placebo was used so patients were blinded.	Unclear.	Adequate; all participants were reported on; however, it is unclear if patients participated more than once in the trial.	No reporting on live birth rate or adverse outcomes or side-effects.	
Moraloglu *et al*., 2010	Inadequate as randomization was by week day.	Unclear.	Patients were blinded as placebo infusion was used.	Unclear.	Adequate as all participants were reported on.	No reporting on live birth rate or ectopic pregnancy.	Exclusion of endometriosis and recurrent implantation failure patients may have biased results.
Ng *et al.*, 2014	Adequate method of randomization by computer-generated random sequence in	Adequate by use of sealed enveloppes and research nurse not	Patients were blinded as placebo infusion was	Yes as codes of treatment and control groups were not revealed until	Adequate as all participants were reported on.	All clinically relevant outcomes were reported.	

	blocks of 10.	involved in the study.	used.	after completion of study and analysis.			
Ohl *et al*., 2002	Adequate; Randomization by third party using randomly permuted blocks of four with stratification.	Adequate allocation concealment as allocation was by randomization service.	Patients were blinded as placebo patches were used.	Unclear	Inadequate as only less than 50% of the target sample was recruited and reported on.	All relevant outcomes are reported on.	The study is underpowered as only less than 50% of target sample was recruited.
Pinheiro *et al*., 2003	Adequate; draw of lots using prepared randomization table.	Unclear	Inadequate blinding as the control arm did not receive placebo.	Unclear	Inadequate as 33 patients (15%) were excluded from analysis after randomization as they stopped active treatment due to side effects.	No reporting on the live birth rate, adverse outcomes (such as ectopic pregnancy rate/multiple pregnancy rate). Insufficient reporting on side-effects of medications.	Insufficient sample size and large drop out rate may have introduced bias.
Rabiee *et al*,. 2011	Unclear method of randomization.	Unclear.	No; blinding of patients.	No; blinding of assessors.	Adequate reporting of all participants.	Only biochemical pregnancy and side-effects reported No reporting on other clinically relevant	

						outcomes.	
Sohrabvand *et al*., 2014	Unclear method of randomization.	Unclear.	No; blinding of patients as no placebo was used.	No clear evidence of blinding of assessors.	Reporting on all included patients.	No reporting on any clinically relevant outcome such as live birth rate or clinical pregnancy rate. Also no reporting on adverse outcomes such as spontaneous abortion rate or ectopic pregnancy rate; however, this was a pilot study.	
Sohrabvand *et al*., 2009	Unclear method of randomization.	Unclear	No; blinding of patients as no placebo used in the control arm; however, the study claims double blinding.	No clear evidence of blinding of assessors.	Adequate reporting on numbers enrolled in the study (*n* = 66); however it is unclear why a number of the recruited (*n* = 356) patients were withdrawn.	No reporting on any clinically relevant outcome such as live birth rate or clinical pregnancy rate; also no reporting on adverse outcomes such as spontaneous abortion rate or ectopic pregnancy rate.	Number recruited at the time of the trial was 352 patients but the number reported on in the trial is 66 patients. The dose of indomethacin is very low and of

							hyoscine is large.
Tsirigottis *et al*., 2000	Unclear method of randomization.	Unclear .	Unclear if blinding was done.	Unclear if blinding was done.	Adequate reporting on all patients.	No reporting on live birth and on side-effects or adverse outcomes.	Small sample size with reduced power.
Shaker *et al*.,1993	Unclear method of randomization.	Unclear.	Blinding of patients achieved.	Blinding of assessors.	Adequate reporting of all patients.	No reporting on live birth, untoward outcomes as spontaneous abortion and ectopic pregnancy rates.	
Zargar *et al*., 2013	Unclear method of randomization	Unclear.	No; blinding of patients achieved.	No blinding of assessors as there was no placebo.	Adequate reporting on all patients participating in the trial.	No reporting on live birth, untoward outcomes as spontaneous abortion and ectopic pregnancy and multiple pregnancy rates.	

Table 3: GRADE summary of evidence table for the effect of uterine relaxants compared with placebo or no intervention in the peri-implantation period for patients having assisted reproduction treatment.

Quality assessment							*Number of patients*		*Effect*			
Number of studies	*Study design*	*Risk of bias*	*Inconsistency*	*Indirectness*	*Imprecision*	*Other considerations*	*Uterine relaxants*	*Placebo or no intervention*	*Relative (95% CI)*	*Absolute (95% CI)*	*Quality*	*Importance*
Live birth (follow up: 10 months)												
4	Randomized trials	Serious[a]	Serious[b]	Not serious	Serious[c]	None	234/687 (34.1%)	214/630 (34.0%)	RR 1.10 (0.88 to 1.38)	34 more per 1000 (from 41 fewer to 129 more)	⊕◯◯◯ Very low[a,b,c]	Important
Clinical pregnancy rate (follow up: 5 months)												
13	Randomised trials	Serious[a]	Serious[b]	Not serious	Serious[c]	None	564/1465 (38.5%)	442/1295 (34.1%)	RR 1.19 (0.98 to 1.44)	65 more per 1000 (from 7 fewer to 150 more)	⊕◯◯◯ Very low[a,b,c]	Important
Spontaneous abortion rate (follow up: 6 months)												
9	Randomised trials	Serious[a]	Serious[b]	Not serious	Serious[c]	None	79/499 (15.8%)	86/421 (20.4%)	RR 0.79 (0.57 to 1.08)	43 fewer per 1000 (from 16 more to 88 fewer)	⊕◯◯◯ Very low[a,b,c]	Important
Ectopic pregnancy (follow up: 12 weeks)												

Quality assessment							*Number of patients*		*Effect*		*Quality*	*Importance*
Number of studies	*Study design*	*Risk of bias*	*Inconsistency*	*Indirectness*	*Imprecision*	*Other considerations*	*Uterine relaxants*	*Placebo or no intervention*	*Relative (95% CI)*	*Absolute (95% CI)*		
3	randomised trials	serious[a]	serious[b]	not serious	serious[c]	publication bias strongly suspected [4]	11/271 (4.1%)	13/255 (5.1%)	RR 0.80 (0.36 to 1.75)	10 fewer per 1000 (from 33 fewer to 38 more)	⊕◯◯◯ Very low[a,b,c,d]	Important
Multiple pregnancy rate (follow up: 10 months)												
4	Randomised trials	Serious[a]	Serious[b]	Not serious	Serious[c]	Publication bias strongly suspected [d]	86/319 (27.0 %)	85/270 (31.5%)	RR 0.89 (0.70 to 1.14)	35 fewer per 1000 (from 44 more to 94 fewer)	⊕◯◯◯ Very low[a,b,c,d]	Important

[a]Inadequate allocation concealment and blinding.
[b]significant clinical and statistical heterogenity.
[c]wide confidence interval crossing unity line.
[d]small number of studies included.

MD, mean difference; RR, relative risk .